

# An Instrument for the Study of Blood Coagulation and Lysis\*

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## Introduction

THE END POINT of all tests of blood coagulation is the formation of fibrin. Indeed, when fibrinogen is absent from a test system, it must be supplied as an indicator substance. While it is often convenient to study clotting in whole blood, the presence of cellular elements serves to obscure the reaction, because all the important factors of coagulation are in the plasma (the platelets generally remaining with this fraction).

A fibrin clot, like beauty, lies in the eye of the beholder. Observers differ greatly in ability to distinguish the changes that herald the start of fibrin formation. Because of the difficulties encountered in attaining accurate and reproducible results, many tests have remained within the exclusive domain of the special coagulation laboratory. However, as medical practice has progressed, there has developed an increasing need for more widespread availability of those tests which are valuable in the diagnosis of hemorrhagic conditions, in following and controlling the effects of drug therapy, and in aiding research studies.

The transformation of fibrinogen into fibrin is accompanied by alterations in many of the physico chemical properties of blood or plasma. Methods which measure viscosity, surface tension, optical, electrolytic, or other properties may be used to detect the onset of, and follow the progress of, this transformation. Although instruments and

methods differ greatly, careful analysis shows that all yield much the same information.<sup>5</sup> While the change that occurs in any particular property of blood during clotting may well reflect the fibrinogen-fibrin conversion, the absolute value of that property usually has little to do with the reaction, since, within broad limits, coagulation is independent of such properties. A simple explanation for the basic similarity of the results obtained by different methods is provided by existing knowledge of the physiology of coagulation. This should not be needlessly confused by a new terminology concerned with the firmness, elasticity, viscosity, resistance, capacitance, etc., of the clot.

A number of instruments for doing clotting studies are available. None of these is capable of performing the entire spectrum of coagulation studies, and the restrictions of some are marked. Having been unsuccessful in finding adequate instrumentation for their own clinical and research purposes, the authors formulated their criteria of what an ideal instrument should do and, using these as a guide, designed and constructed one. This photoelectric instrument (called a coagulograph) has proved simple to use, rugged, reliable, and versatile. In addition to furnishing continuous automatic recording of rapid or slow coagulation reactions under controlled conditions, it affords a measure of the amount of fibrin formed in the reaction and provides a unique and useful method of detecting, following, and quantitating fibrinolysis.

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## The Coagulograph

The instrument (Fig. 1, 2) is contained in a metal cabinet and consists of a power supply, three photoelectric reaction chambers mounted in a constant temperature housing, three recorders, and the necessary circuitry. Each photoelectric reaction chamber, with its light, circuit components and corresponding recorder, constitutes a functional unit referred to as a channel. The three channels, from left to right, are designated 1, 2, and 3. The power supply converts 110 volt, 60 cycle, alternating current into full wave rectified direct current and automatically compensates for variations in load and line voltage. It provides current for the lamps and photoelectric bridge circuits. The heating unit and drive mechanisms of the recorders are run by line current. The constant temperature housing consists of a rectangular block of aluminum which is fixed to the cabinet and serves as a base for mounting the superstructure and contents of the three reaction chambers. Inserted into the block are a cartridge heater and a thermoswitch for temperature regulation.

The two reaction chambers on the left side of the block (channels 1 and 2) are identical. Each is designed to receive a specimen horizontally and transmit light through it in a vertical direction. The light source, a small incandescent lamp, is mounted in the block in a removable socket. The light beam passes through an aperture in the base of the chamber and is focused on a photocell, mounted above in a horizontal supporting partition. A hinged cover provides light-tight closure of the chamber. The reaction chamber on the right (channel 3) accepts a specimen vertically and transmits light through it horizontally. The superstructure surrounds a square opening in the block. Both lamp and photocell are mounted in the block, the lamp in front, focusing through an aperture on the photocell behind. Removable adap-

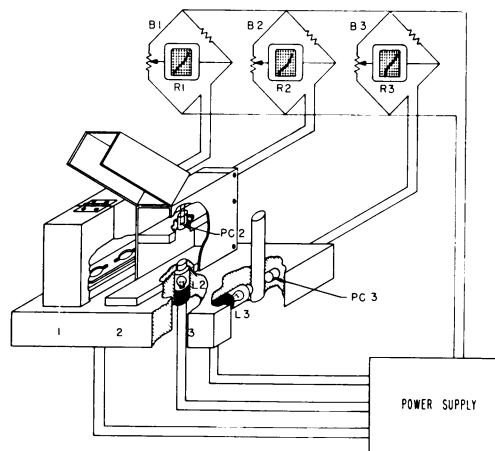


FIG. 1. Schematic diagram of the coagulograph showing the three channels (1, 2, 3), lamps (L), photocells (PC), bridge circuits (B) and strip recorders (R). For simplicity, the null balance meter is not shown.

tors are made to slide into and out of this chamber vertically. Each adaptor has a vertical recess for a test tube and aligned openings, front and rear, which control the size of the light beam and assure encompassment of the beam by the specimen. A removable light cover fits over the chamber.

The current to the lamps is constant (approximately 170 ma) and about two-thirds their rated capacity. The light-sensing elements are photoconductive cells of small size and cylindrical shape. Each photocell comprises one arm of a bridge circuit. The

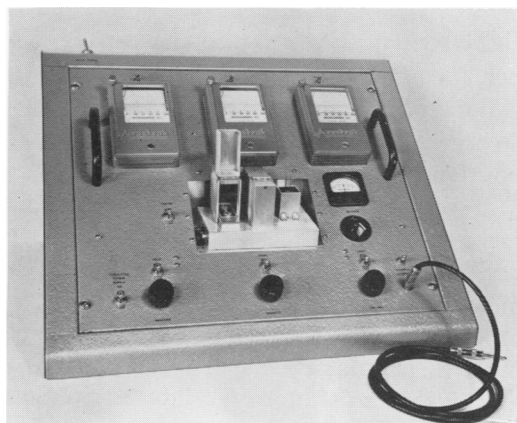


FIG. 2. The coagulograph.

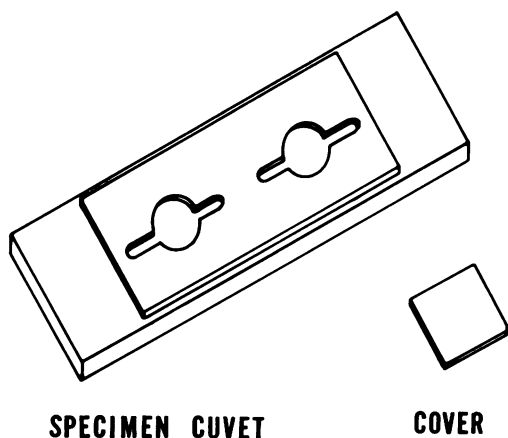


FIG. 3. Design of specimen cuvetts.

other arms are a fixed resistor and a variable resistor, the latter serving to balance the bridge. Voltage across the bridge is kept constant (about 15 volts). The output current of the bridge passes through another variable resistor (sensitivity control) to a zero-center microammeter, which by means of a rotary switch serves, in turn, as a common balance indicator for each of the three channels. This meter protects the more delicate recorders from excessive current due to gross imbalance of a bridge. A separate switch shunts current flow from the balance meter to the recorder for each channel and simultaneously turns on the recorder drive mechanism. A phone jack permits shunting of channel 3 to a separate recorder. The recorders are small strip chart recorders which use 6-cm.-wide paper, have variable paper speeds and access windows for marking the record. A decrease in light transmission is shown as a positive deflection from the baseline.

A special cuvet (Fig. 3) holds specimens for vertical light transmission in channels 1 and 2. The cuvet may be made of methylmethacrylate plastic (Plexiglas), glass, or other rigid transparent material. Each cuvet has two circular specimen wells placed so that insertion of a cuvet into a reaction chamber automatically aligns one well with the light path; reversal of the cuvet aligns

the other well. Each well has two side openings and a square cover which fits over its circular portion. A specimen is introduced through one of the feeder slots while the cover is in place, or the well is filled and the cover applied. The side openings prevent trapping of air bubbles. The wells are made in different depths, 0.5, 1.0, and 3.0 mm. The volume of fluid required to fill each well is about 0.04, 0.08, and 0.2 ml., respectively.

### Procedure

Slow reactions, and all whole blood reactions, are conducted in channels 1 and 2. Channel 3 is used for either slow or fast reactions. For recording the latter, channel 3 is jacked out to a DC amplifier or an ECG. A plane-sided test tube with a 5 mm. light path is used and requires a total fluid volume of 0.3 ml. The instrument is ready for use after a short warm-up period and can be left *on* for hours or days. Standardization is accomplished by setting the sensitivity control of each channel so that a reference solution (or filter) produces a constant reduction of light transmission. Once set, standardization remains stable for weeks.

The special cuvetts, because of their small depth, permit adequate light transmission through whole blood and minimize the effect of sedimentation of the red cells. The shallowness of the well and its large surface area are sufficient to prevent clot retraction, and an increase in light transmission after the completion of fibrin formation signifies dissolution or lysis of the clot.

Retraction of the clot is only seen with plasma specimens observed in channel 3, since the cuvetts used in channels 1 and 2 prevent retraction. After the completion of fibrin formation retraction is manifest as a sudden, irregular deflection which may be positive or negative, depending upon whether the retracting clot, with its increasing density, continues to encompass the

light beam or pulls away from it, permitting direct passage of light through serum.

Blood samples are obtained and handled with the meticulous care customary in coagulation work. Plastic (polystyrene) syringes, test tubes, transfer pipets and cuvetts are used to provide nonwetable surfaces. Glass activation is accomplished by placing whole blood in a glass cuvet or by adding a measured amount of small glass particles to citrated whole blood or plasma. Reactions are routinely run at 37° C. The anticoagulant usually employed is 0.1 M. sodium citrate (blood to citrate ratio, 9:1). In specimens with very high or low hematocrit values, correction of the amount of citrate added to 9 volumes of blood is made according to the method of Nygaard<sup>9</sup> to provide a constant citrate concentration per ml. of genuine plasma. Platelet-rich plasma is obtained by centrifugation at 1,500 rpm for five minutes. Recalcification is with 0.1 M. calcium chloride; 0.1 ml. for each 0.3 ml. of plasma or 0.05 ml. for each 0.3 ml. of citrated whole blood. For prothrombin time determinations and other rapid reactions, conventional reagents are employed. Euglobulin is prepared by the method of Buckell (1958), as cited by Biggs and Macfarlane.<sup>1</sup>

Certain operations are common to the use of all channels. The filled specimen container (cuvet for channels 1 and 2; test tube for channel 3) is introduced into a chamber. The rotary switch is turned to that channel and the meter balanced by use of the appropriate potentiometer. The recorder switch for the channel is then turned on. Plasma recordings are started a little above the zero level to bring the baseline away from the edge of the paper. Recordings of whole blood are started in the middle of the paper span to allow for the initial negative deflection caused by sedimentation of the red cells. These adjustments of the baseline are accomplished by the potentiometer immediately after the recorder is started. When a tracing appears to be go-

ing off the paper, the potentiometer is used to bring it back to a new low level. These manipulations do not appreciably affect the linearity of the tracing.

Citrated plasma and citrated whole blood are recalcified in a test tube before introduction into a specimen well. Recording is started 30 seconds after recalcification. With whole blood, the time from venepuncture to the start of recording is added to the latent period. Routinely 0.5 mm. cuvetts are used for whole blood; 1.0 mm. cuvetts for citrated whole blood, and 3.0 mm. cuvetts for plasma or other relatively transparent specimens.

In channel 3, slow plasma reactions are conducted as above. For fast reactions, the prothrombin time determination is taken as an example. The test tube containing the thromboplastin-plasma mixture is introduced and the meter balanced. The chart drive of the recorder is turned on. The calcium solution is blown in, and the event marker button pushed. The test tube is quickly removed, shaken twice, and reinserted. The meter is restored to balance, and the bridge current switched to the recorder. The end point is a sudden steady movement of the curve up scale.

## Results

Coagulograms can be obtained on whole blood, recalcified whole blood, native plasma, and recalcified plasma (Fig. 4). Citrated whole blood gives a curve which progressively declines to reach a plateau—this simply reflects sedimentation of the red cells. When citrated whole blood is recalcified, the fall in the curve is terminated by an upward deflection, which marks the onset of fibrin formation. From this point on, the curves of recalcified whole blood and plasma are essentially similar, the differences in amplitude being due to differences in depth of the specimen (1.0 mm. well used for recalcified whole blood and 3.0 mm. for plasma) and in alterations in the light reaching the photocell through

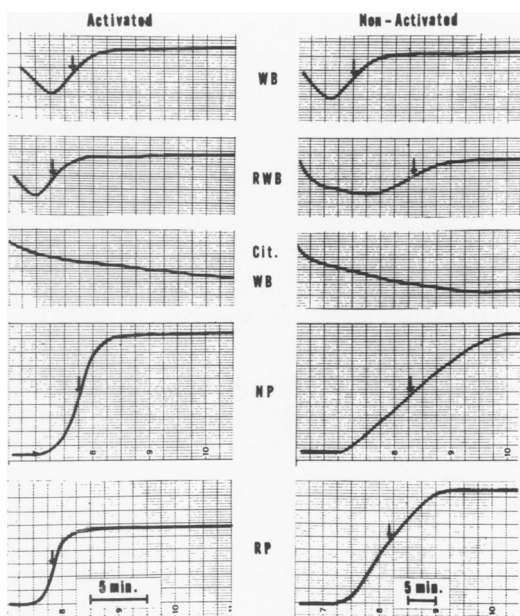


FIG. 4. Coagulograms of a normal subject. A positive deflection represents diminished light transmission. Vertical scale units are microamperes. Horizontal (time) scale is in minutes as shown. Note that recording speed for curves in right-hand column is one-half as fast as for those in left-hand column. *Activated* indicates exposure to a glass surface. *Nonactivated* indicates blood has been handled throughout in nonwettable (in this instance, plastic) containers. WB, whole blood; RWB, recalcified whole blood; Cit WB, citrated whole blood; NP, native plasma; RP, recalcified plasma. Arrows indicate half-way point in fibrinogen to fibrin conversion.

the redder and denser whole blood at the same sensitivity setting used for plasma. Whole blood placed directly into a cuvet (0.5 mm. depth) from finger puncture or syringe produces a curve very like that of recalcified whole blood.

When blood is collected and handled throughout in nonwettable containers (non-activated column in Fig. 4), the onset of fibrin formation is delayed, and the time required for its completion is prolonged, in comparison to the onset and speed of fibrin formation in the same blood after exposure to glass surfaces (activated column in Fig. 4).

A schematic drawing of a plasma coagulogram is presented in Figure 5. It starts as a horizontal line which, after a period of time, curves upward and then levels off to run more or less horizontally again. Unless lysis or retraction occurs, the curve continues indefinitely at that level. Lysis is manifested by a downward turn of the tracing. Point F marks the onset of fibrin formation, Point C its completion, and Point R the start of lysis. Period  $r$  is the reaction or latent period before clotting begins;  $k$  is the period of fibrin formation;  $l$ , the time after the completion of fibrin formation to the onset of lysis; and  $t$ , the duration of lysis;  $a$ , in units of decreased

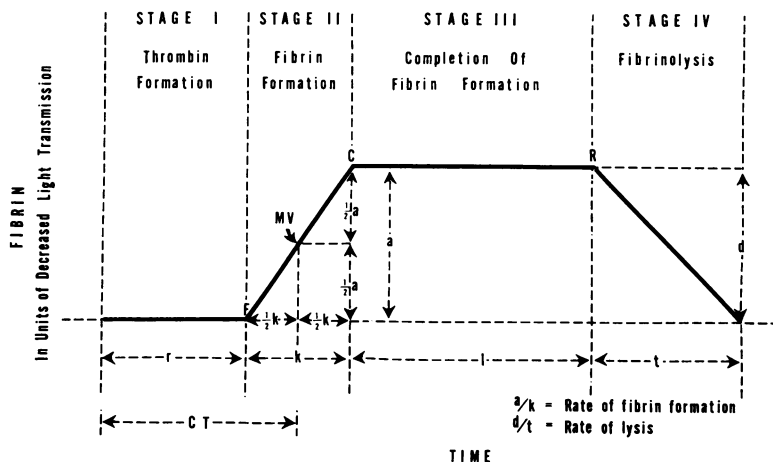


FIG. 5. Schematic representation of a plasma coagulogram, illustrating significance of various geometric stages of the curve in terms of fibrin formation and lysis and presenting a method of designating points, time intervals, and changes in amplitude which are of interest and importance in making quantitative determinations.

light transmission (microamperes), is proportional to the amount of fibrin formed and d, conversely, to the amount of fibrin lysed. MV is the midpoint of fibrin formation and coincides with the point of maximal velocity of fibrin formation. The time (CT) from the start of the curve to MV roughly corresponds to the clotting time as determined by conventional visual means.

In Figure 6 is shown the correlation between the amplitude (a in Fig. 5) of coagulograms of recalcified plasma and the fibrinogen content of these plasmas as determined by the method of Parfentjev.<sup>4, 10</sup> Figure 7 shows a similar correlation between the amplitude of prothrombin time curves and the fibrinogen content of plasma. A commercial plasma was used as the reference standard for all tests—the sensitivity of the channel being set to give an amplitude of 26 microamps on the recalcified plasma curve and 15 mm. on the prothrombin time curve. The correlation is sufficiently linear to allow direct approximation of the fibrinogen content of a plasma

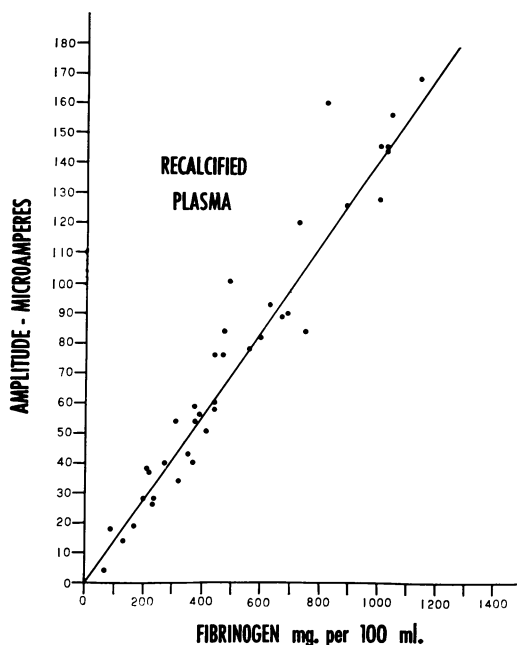


FIG. 6. Correlation between fibrinogen content (Parfentjev method) and the amplitude of the coagulogram of recalcified plasma.

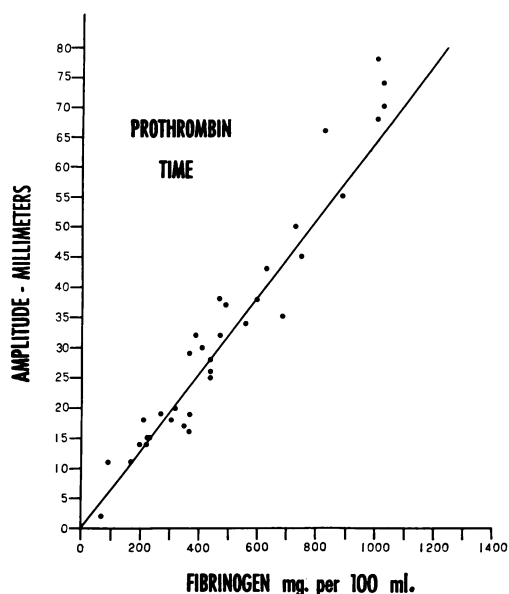


FIG. 7. Correlation between fibrinogen content (Parfentjev method) and amplitude of the prothrombin time coagulogram (Channel 3).

sample from the coagulogram or prothrombin time curve. The amplitudes of coagulograms produced by clotting plasma with thrombin or doing a partial thromboplastin time test are equally well related to fibrinogen content. Coagulograms of whole blood and recalcified whole blood resemble their respective plasma curves but, as might be expected, do not provide as accurate or as reproducible measurements of the amount of fibrin formed. The amplitudes of coagulograms of fibrinogen solutions clotted with thrombin and of plasma clotted by calcium, thrombin, or thromboplastin-calcium are directly proportional to the degree of dilution of the original fibrinogen solutions or plasma (Fig. 8).

Turning down a curve to permit registration of a coagulogram of high amplitude does not alter the linearity of the recording appreciably (Fig. 9a, b). By varying sensitivity, the amplitude of coagulograms can be expanded or contracted (Fig. 9c). As illustrated in Figure 9d, the amplitude of a plasma coagulogram is proportional to the depth of the specimen.

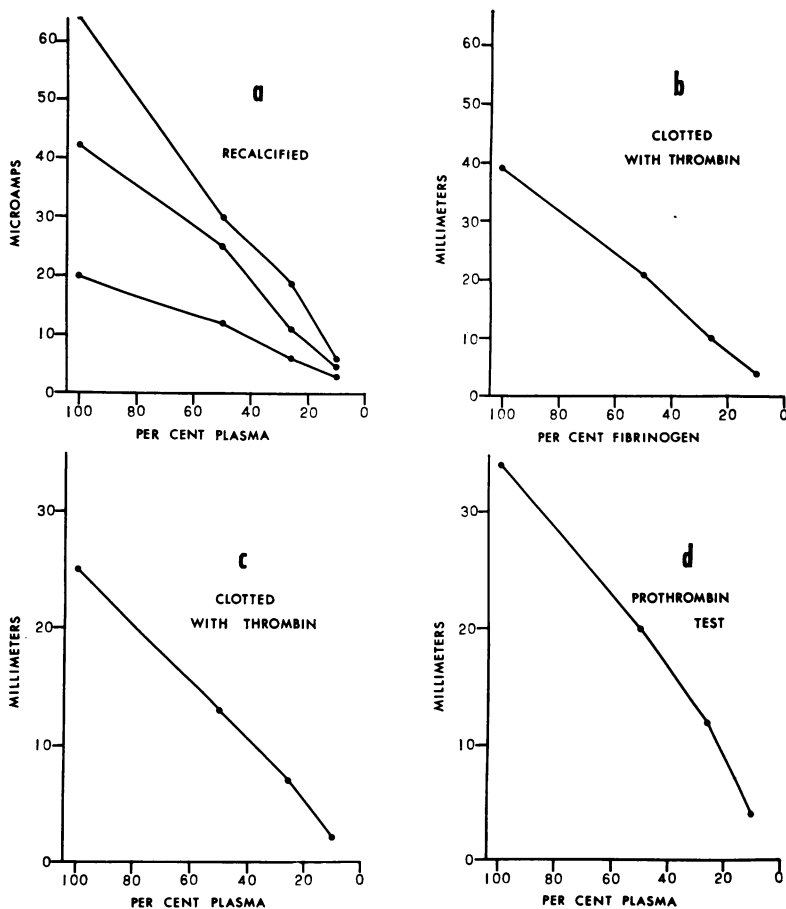


FIG. 8. Effect of saline dilution on amplitude of various coagulograms: a. Recalcified plasma—sensitivity varied to give three different initial values for undiluted plasma. b. Fibrinogen solution (300 mg./100 ml.) clotted with thrombin. c. Citrated plasma clotted with thrombin. d. Prothrombin time test (one stage) on citrated plasma.

If the concentration of thrombin in a potent solution is serially reduced by dilution, a point is reached beyond which further dilution causes progressive lengthening of the latent period and slowing of the speed of fibrin formation when the same aliquot of thrombin solution is added to citrated plasma (Fig. 10, upper right). Prolongation of the thrombin-clotting time of citrated plasma to which heparin has been added is related to the concentration of heparin in the plasma (Fig. 10, lower right). The effect of heparin on the thrombin-clotting time of citrated plasma can be abolished by protamine sulfate or hexadimethrine bromide, and the amount of these substances required to restore the thrombin-clotting time to control values is proportional to the amount

of heparin present (Fig. 14B). In addition to the titration curves already discussed, Figure 10 shows a graph of prothrombin time determinations on various saline dilutions of normal plasma (upper left) and the prothrombin times of the same plasma when the thromboplastin is serially diluted (lower left). The latent periods of coagulograms are plotted as prothrombin times.

Figures 11 and 12 furnish a coagulographic demonstration of a variety of coagulation defects. For the most part, the coagulograms and their accompanying legends furnish sufficient explanation. The hypocoagulability of the blood of the patient with hemophilia (Fig. 11a, b) and the patient with thrombocytopenic purpura (Fig. 11c, d) is just as evident in plastic as

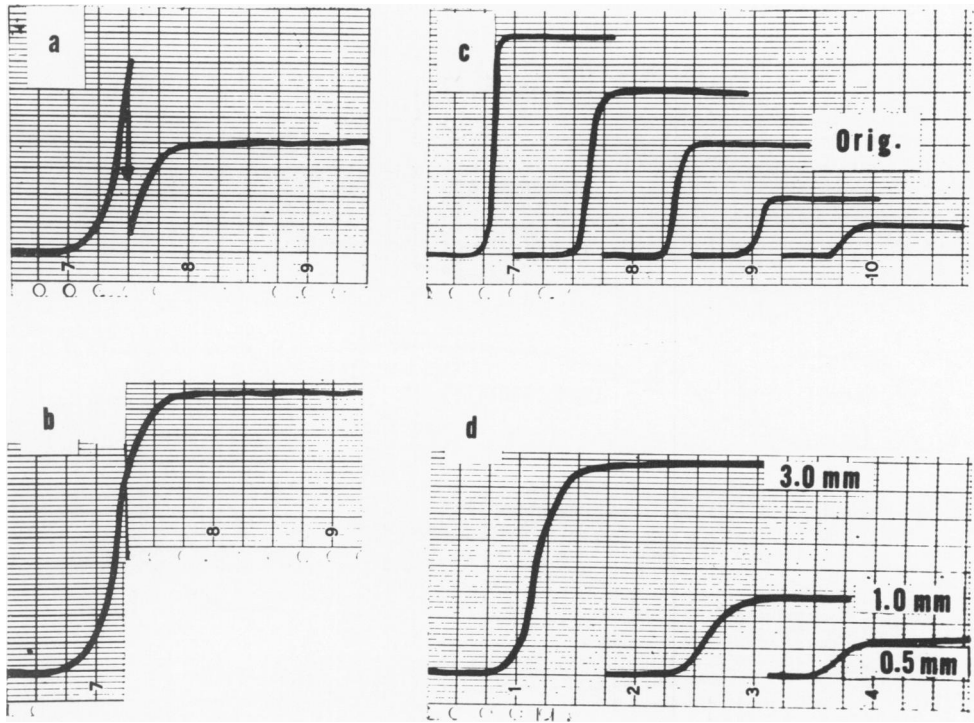


FIG. 9. Coagulograms of recalcified plasma which illustrate linearity of variations in amplitude resulting from changes in sensitivity, or in depth of specimen. a. Plasma with high fibrinogen content—curve turned back as indicated by arrow to keep recording on paper. b. Same curve as in a. Record has been cut along line of arrow and right-hand portion moved up to join two portions of curve and show its continuity. c. Plasma with an amplitude of 40 microamp., showing recording at 25, 50, 150, and 200% of original sensitivity. d. Plasma—three different cuvetts used, with specimen depths of 0.5, 1.0, and 3.0 mm., respectively.

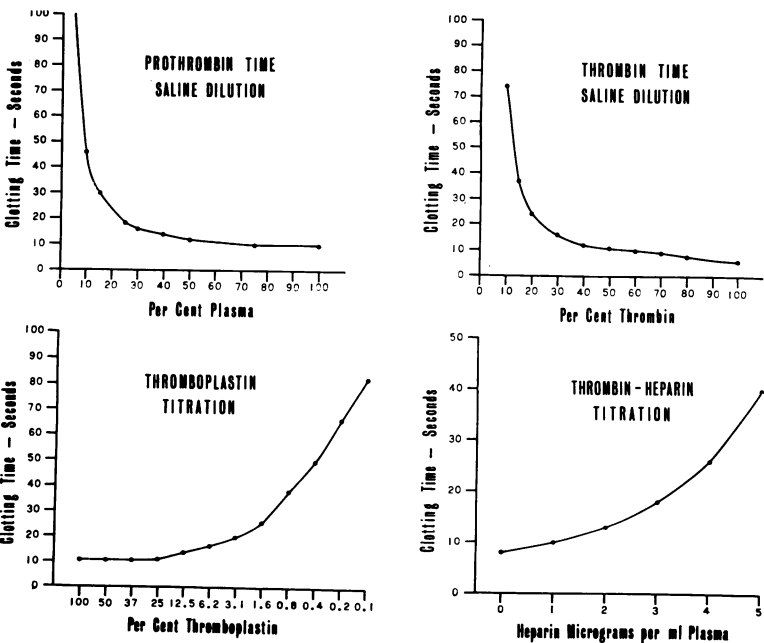


FIG. 10. Dilution and titration curves plotted from data obtained from coagulograms of citrated plasma.

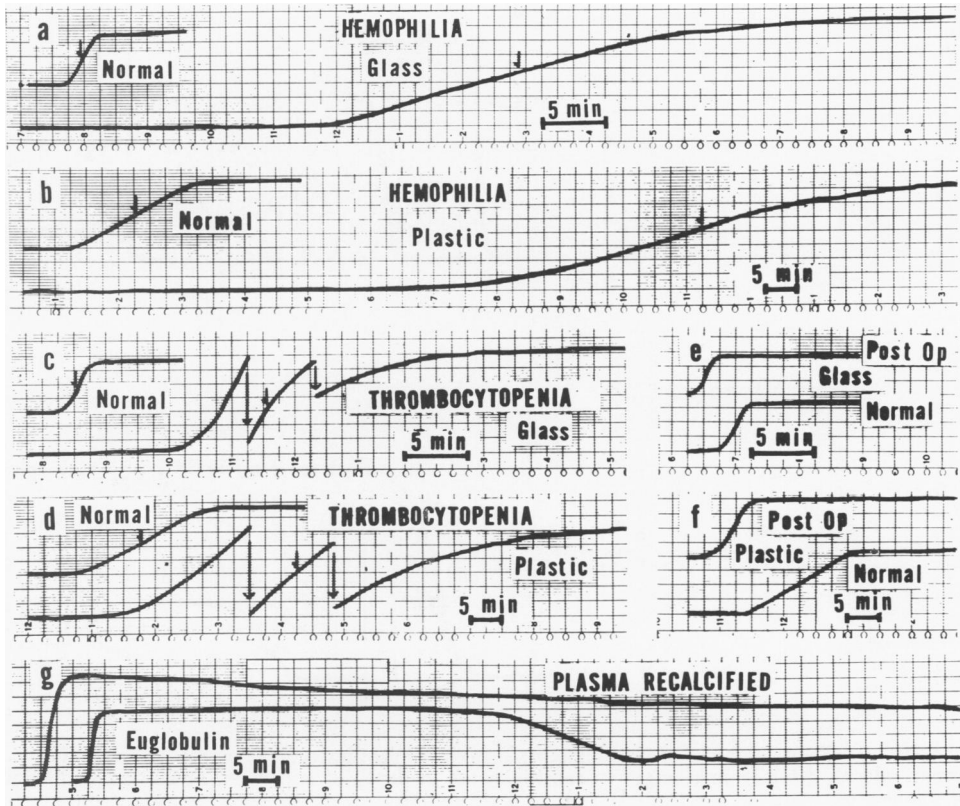


FIG. 11. Abnormal coagulograms: a, b. Recalcified plasma curves of a young hemophiliac with moderately severe spontaneous hemorrhages. c, d. Recalcified plasma curves of a 68-year-old man with thrombocytopenic purpura. Platelet count 40,000. Note hyperfibrinogenemia. e, f. Recalcified plasma curves of a 41-year-old man two hours after elective hemigastrectomy and vagotomy for duodenal ulcer. g. Coagulograms of a 48-year-old man with recurrent nasal hemorrhage. Recalcified plasma curve (above) shows slow fibrinolysis. Euglobulin curve (below) shows faster lysis. Patient had no other demonstrable coagulation defect.

in glass, while the hypercoagulability of the postoperative patient (Fig. 11e, f) is more obvious in plastic. It should be noted that recording speed for coagulograms in plastic is one-half that for those in glass. Deficiencies in clotting factors which produce prolongation of the one-stage prothrombin time (Fig. 12b) can also produce abnormalities in the recalcified plasma coagulogram (Fig. 12a, c). Coagulograms depict rather well the end results of the multiple defects that may occur in the blood of patients with cirrhosis of the liver. As shown in Fig 12d, there may be hypo-coagulability, hypofibrinogenemia, and evidence of fibrinolytic activity. Figures 11 and 12 also demonstrate, quite adventiti-

ously, that there is no direct relationship between the fibrinogen content (except when critically low) and the coagulability of recalcified plasma. In four instances of hypocoagulability (Fig. 11a, c) (Fig. 12a, c), there is hyperfibrinogenemia, while in one instance of hypercoagulability (Fig. 11e, f), the fibrinogen concentration is within normal limits. The use of the coagulograph in the differential diagnosis of a coagulation defect is shown in Figure 13.

The ability of protamine sulfate to abolish the effect of heparin *in vivo* and *in vitro* is shown in Fig. 14a, b. Following the intravenous injection of heparin, the plasma of the subject (dog) was rendered incoagulable (b in both figures). The amount

of protamine sulfate required to return the thrombin-clotting curve to control levels was determined by titration (Fig. 14b, c), and this concentration of protamine, when added *in vitro* to citrated plasma, produced, on recalcification of the plasma, a

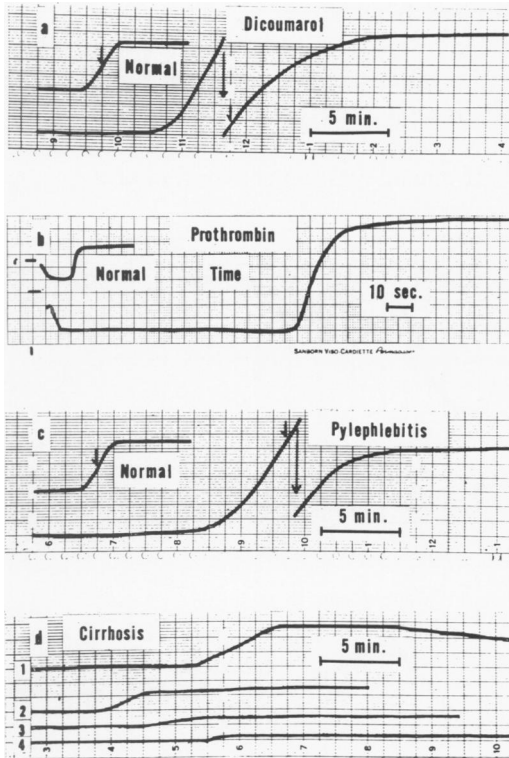


FIG. 12. Abnormal coagulograms: a. Recalcified plasma curve of a 68-year-old man with arteriosclerotic heart disease, congestive failure, and thrombosis of right common iliac artery. Patient had been on long-term dicoumarol therapy following a previous myocardial infarction and had been taking excess dicoumarol prior to admission. Fibrinogen level is high and coagulation considerably delayed. Platelet count 210,000. b. Recording of the prothrombin time of same patient. c. Recalcified plasma curve of a 37-year-old man with pylephlebitis and liver abscesses. Fibrinogen concentration 850 mg./100 ml. Prothrombin time 25% of normal. d. Recalcified plasma coagulograms of patients with Laennec's cirrhosis of liver. 1. Hematemesis from varices (49-year-old man). Clotting prolonged, fibrinogen low normal and fibrinolysis apparent. Prothrombin time 35%. Platelet count 195,000. 2. Portacaval shunt 3 months previously for variceal hemorrhage (39-year-old man). 3. Hepatic coma, PT 17%, chronic anemia but no clinical hemorrhage (69-year-old man). 4. Ascites, anemia, and jaundice (45-year-old man). Prothrombin time 27%. Platelet count 150,000. Died in hepatic coma. No clinical evidence of bleeding.

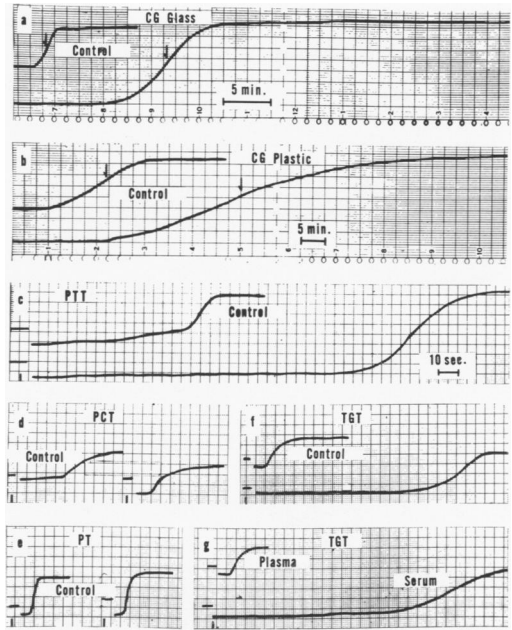


FIG. 13. Coagulographic diagnosis of the coagulation defect of a 17-year-old boy with a history of excessive bleeding following minor cuts. No spontaneous hemorrhages. Positive family history. Bleeding time normal. Tourniquet test negative. Platelet count 220,000. a, b. Coagulograms of recalcified plasma in glass and plastic, respectively, each showing defect in clotting. c. Partial thromboplastin time. d. Prothrombin consumption time. e. Prothrombin time. f. Thromboplastin generation test. g. Thromboplastin generation test showing correction of patient's defect by substituting normal plasma for patient's plasma and failure of correction by normal serum. Diagnosis—hemophilia. Time scale in c also applies to d, e, f, and g. Serum derived from whole blood.

coagulogram similar to the control or baseline tracing. After the intravenous administration of protamine, both the thrombin-clotting time and recalcified plasma coagulograms were restored to normal.

Fig. 15 summarizes in a series of coagulograms the manner in which fibrinolysis can be detected and quantitated in a subject (dog) whose blood has been rendered incoagulable by heparin. The reduction of fibrinolytic activity by administration of epsilon-aminocaproic acid (EACA) is also shown (d)—again the coagulability of the plasma being restored by *in vitro* neutralization of heparin. As a final phase, the heparin effect was abolished *in vivo* (e).

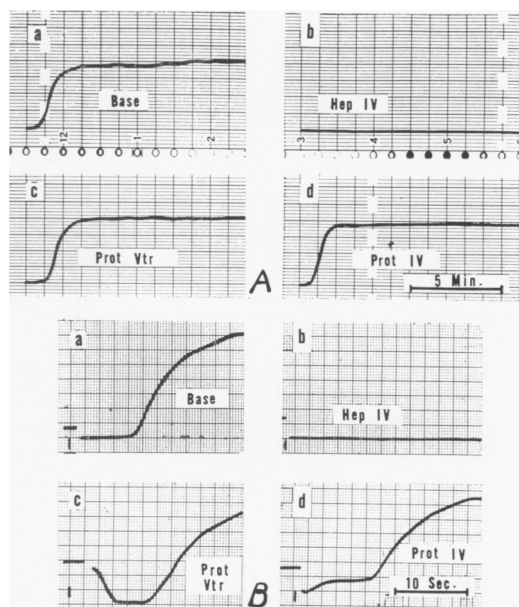


FIG. 14. Heparin neutralization *in vivo* and *in vitro* (Vtr.). Dog. A. (Above) Coagulograms of recalcified plasma. a. Base line tracing. b. After heparin IV. c. Same plasma as b with protamine sulfate added before recalcification. d. Plasma sample after protamine sulfate administered IV. B. (Below) Thrombin titration a, b, c, & d—same as corresponding letters above.

Although not shown, the amounts of hexadimethrine bromide required for *in vitro* and *in vivo* neutralization of heparin were found by thrombin-clotting time titration (as in Fig. 14c).

The concentrations of streptokinase and two different commercial preparations of human fibrinolysin needed to produce comparable degrees of fibrinolysis in pooled plasma from normal subjects are shown in Figure 16. In repeated tests, the relative amounts of the drugs required to produce similar lysis were fairly constant with different normal plasmas.

### Conclusions and Discussion

To a considerable degree, the satisfactory performance of the coagulograph is due to the use of photoconductive cells which do not show fatigue, have a good linear response, and permit ready control of sensitivity. The use of small bulbs which run at

less than rated currents, eliminates the problem of heat production and assures long life. The current flow, occasioned by introducing a sample into the light path of a photocell, is balanced to zero by means of the bridge circuit, and only the changes which occur during coagulation are registered. The effects of differences in the color, transparency, or other optical properties of specimens, and even the effects of slight smudging or small scratches on the cuvetts are cancelled out.

If one adopts the convention of depicting

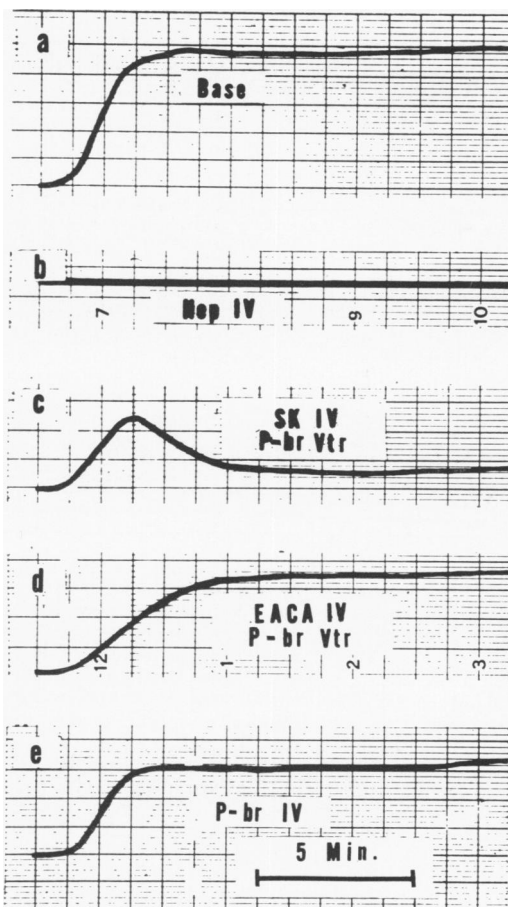


FIG. 15. Streptokinase-induced fibrinolysis in the heparinized dog. Recalcified plasma curves. a. Base line curve. b. After heparin IV. c. After SK IV in the heparinized dog. Heparin neutralized *in vitro* by polybrene. d. SK induced fibrinolysis reduced by EACA IV; heparin effect neutralized by polybrene added to plasma *in vitro*. e. Heparin effect in dog neutralized by polybrene IV.

all clotting curves in the same fashion, that is, horizontally, with the latent period to the left, and the main curve of change as an upward deflection from the baseline, then, regardless of the property measured or the detecting or recording apparatus used, their fundamental similarity is more evident. The resemblance of coagulograms to viscosity curves and thrombelastograms,<sup>5</sup> and especially to the photoelectric tracings of Elliott<sup>3</sup> and Nygaard,<sup>9</sup> is obvious, and contributes convincing evidence of the validity of the coagulographic method.

In the development of the coagulograph, the following findings of Nygaard were confirmed:

Within limits, variations in the intensity of light reaching the photocell through a specimen do not alter the time relationships of the coagulogram significantly. The degree of illumination does, however, have a marked effect on amplitude, and for this reason, must be standardized, if the coagulogram is to be used to indicate the amount of fibrin formed.

The use of light filters, although perhaps theoretically desirable, does not result in demonstrable practical benefit.

For photoelectric studies, sodium citrate is preferred to oxalate as an anticoagulant, because the latter causes, on recalcification, an initial slight decrease in light transmission due to precipitation of calcium oxalate crystals.

The velocity of coagulation and the amount of fibrin formed by the recalcification of plasma are not absolute, but relative values and depend on both the citrate and calcium concentration in plasma. Indeed, an excess of calcium has an anticoagulant effect. To achieve comparable results technic must be standardized so that a constant concentration of anticoagulant and recalcifying solution per volume unit of genuine plasma always obtains. For this reason, with but slight modification, the standard technic of Nygaard was adopted.

The clotting of blood or plasma is maximally short at 37° C. and is prolonged at much higher or lower temperatures.

When standard technic is employed, the amplitude of the coagulogram (geometric equivalent of fibrin) is proportional to the amount of fibrin formed.

Replicate coagulograms of recalcified plasma have a coefficient of variation of about 10 per cent.

The velocity of fibrin formation is, to a large

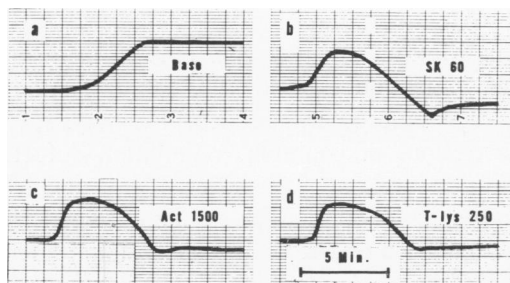


FIG. 16. *In vitro* fibrinolysis induced by three different drugs in pooled plasma from normal subjects—recalcified 3 min. after addition of agent. Amounts of drug (units as specified by manufacturer) selected to produce comparable degrees of lysis. Note characteristic shortening of reaction time caused by all agents. a. Base line curve. b. Streptokinase 60 units/ml. plasma. c. Actase 1500 units/ml. plasma. d. Thrombolylin 250 units/ml. plasma.

degree, determined by the duration of the first phase of coagulation, that is, the latent period. When the latent period is short or long, fibrin formation is correspondingly rapid or prolonged.

The duration of the latent period is, within certain limits, not influenced by the concentration of fibrinogen present in the coagulating system.

Prolonged and/or rapid centrifugation of blood increases the clotting time of plasma; roughly in proportion to the degree of reduction of the platelet concentration.

The spectrophotometric method, described by Losner and his associates,<sup>7,8</sup> for measuring fibrinogen concentration and prothrombin time simultaneously, has been used in our laboratory for a number of years. Coagulographic and spectrophotometric results on the same plasmas correlate very well. The spectrophotometric method has the disadvantage of not being able to measure with any degree of accuracy fibrinogen concentrations of less than 120 mg./100 ml. Ranof and Volk with their co-workers<sup>6,13</sup> worked out the relationship of the erythrocyte sedimentation rate to plasma fibrinogen concentration and pointed out the superiority of the latter over the former as a means of following in a nonspecific way the activity of inflammatory and necrotic reactions. They stressed the clinical value of serial fibrinogen determinations in conditions such as rheumatic fever and myocardial infarction.

Coagulographic results (as shown in various dilution and titration curves, heparin-neutralization experiments, demonstrations of spontaneous and induced fibrinolysis, illustrations of common coagulation defects in patients) are all in excellent general agreement with similar results obtained by coagulation experts with conventional manual-visual technics as presented in standard reference works.<sup>1, 2, 11, 12, 14</sup>

Coagulograms depict fibrinolysis and paracoagulation as clearly as thrombelastograms.<sup>14</sup> The coagulograph detects and follows lysis in fibrinogen solutions clotted by thrombin or in euglobulin preparations clotted by recalcification or thrombin. In active lytic systems, lysis may be apparent in the prothrombin time coagulogram. As a result of lysis, the plasma specimen, under coagulographic examination, frequently becomes more transparent than it was originally (Fig. 16).

Since blood is normally fluid *in vivo*, coagulation and lysis are only potential properties, and all methods of *in vitro* study are unphysiological and arbitrary. The value of a method depends upon the reliability of the end points selected and the degree to which extrinsic factors that influence clotting are controlled and standardized. The transformation of fibrinogen to fibrin actually presents two end points: one, the onset, and two, the completion of fibrin formation. In a fast reaction, these points are close together and sharp; in a slow reaction, farther apart and less clearly delineated. The initiation of fibrin formation may be so insidious that it defies definition as a point in time, or so sudden and evanescent that visual detection is difficult. The coagulation of blood is a dynamic reaction and is best followed by a method which provides continuous automatic recording of the process and affords as much information as possible about the quality of the clot and the kinetics of its formation, retraction, or dissolution.

No instrument can eliminate the vagaries

or complexities inherent in the interaction of intrinsic clotting factors or overcome artifacts induced by faulty methods of collecting or handling blood specimens. Aside from these limitations, the coagulograph constitutes a complete instrumentation system for the study of coagulation and lytic phenomena in blood and other fluids. It enables any competent technician to achieve accurate and reproducible results previously attainable only by specialists. It can, therefore, encourage wider and more confident use of tests of coagulation and lysis which are now seldom done in many laboratories because of the time required and the unreliability of the results. Whether used for clinical or research studies, the coagulograph provides convenience, a saving of technician time, and the accumulation of a body of detailed, recorded data under standard conditions. It also furnishes a means of continuing observation of slow reactions after laboratory personnel have left for the day.

In clinical situations involving hemorrhage or thrombosis in which speed of diagnosis is important, a coagulogram of recalcified plasma gives more information than any other single determination and does so expeditiously. When indicated, additional tests may be performed on the coagulograph, using the same citrated blood drawn for the original study. Coagulograms also provide a convenient and quantitative method for following and assessing the results of therapy. The coagulographic technic is singularly useful in detecting and quantitating fibrinolysis, even in heparinized blood. It promises to have merit in predicting the *in vivo* effect of fibrinolytic enzymes from *in vitro* tests. Coagulograms, because they are graphic representations, are of value as teaching and training aids.

### Summary

During a protracted and rigorous period of testing and in subsequent clinical and research studies, the coagulograph has satis-

fied criteria postulated as necessary for an ideal instrument for coagulation studies. It:

Provides an end-point detector-reaction follower system for all coagulation studies.

Provides recordings which show all phases of coagulation well defined geometrically and useful in quantitating fibrin formation and lysis. Recording is direct, with the tracings always visible, accessible for marking, and removable as required. The records are small enough for easy storage or placement in a hospital chart.

Accepts samples of small size and provides an environment which is temperature controlled, without the mess of a water bath. There is no agitation or disturbance of the specimen after introduction, and neither the detecting nor recording means affects the reaction.

Affords ease and reliability of standardization and adjustable control of sensitivity.

Provides multichannel capability, so that duplicate or other studies may be conducted simultaneously.

Runs off ordinary wall current and compensates automatically for variations in line voltage.

Provides inexpensive, disposable or reusable specimen cuvetts.

Works on physical principles which are generally familiar to laboratory personnel and permits the employment of conventional test methods and reagents. It is workable by any competent technician after a short period of training.

Achieves results comparable in accuracy and reproducibility to those of existing technics.

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